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RELATION OF GREEN LINT TO LINT INDEX IN UPLAND COTTON[1]

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INTRODUCTION

Cotton breeders and producers of cotton have long been interested in obtaining commercial varieties that possess a combination of high lint percentage and other desirable characters. The exact mode of inheritance of lint abundance is not understood, presumably because it is a compound character and each of the functions affecting it is in turn inherently complex and affected by a number of environmental influences. A more thorough understanding of the mode of inheritance of the capacity of a cotton plant to produce a characteristic quantity of lint on the individual seed should be of value to breeders who are working along these lines of improvement.

GREEN-LINT CHARACTER

The cotton fiber, being an extended epidermal cell, is a unicellular structure. The number of fibers arising from the epidermal layer of a single seed of the commercial upland cottons has been estimated as between 10,000 and 20,000. Commercial cotton fibers are white or light cream. In several noncommercial varieties, brown fibers of various shades are found, and at least one noncommercial variety is characterized by green fibers. The green pigment may be seen soon after the fibers begin to thicken and is apparent by 25 days after flowering. As the fibers develop, the color is intensified until the boll opens, displaying the bright-green pigmentation. On exposure to light the color gradually fades to a brownish green. Kerr has found that the green pigment occurs in the cell wall and that this coloration is not present in the protoplasmic contents of the cell.[3]

Harland (*6*)[4] and Ware (*24*) have reported that green lint is determined primarily by a single pair of genetic factors, to which the symbols *Lg*, *lg* have been assigned by Hutchinson and Silow (*9*). Green is incompletely dominant over white, the F_1 being characterized by a reduced amount of color. The F_2 segregation consists of green, intermediate green, and white segregates in a ratio of 1 : 2 : 1. Progenies of the F_2 green and white phenotypes breed true in the F_3, and progenies of the F_2 intermediate-green phenotypes again segregate into three classes. Segregation in the study here presented conformed satisfactorily to the monofactor hypothesis, since a popu-

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[3] The writer is grateful to Thomas Kerr, of this Division, for permission to refer to this heretofore unpublished information.

[4] Italic numbers in parentheses refer to Literature Cited, p. 305.

lation segregating in the ratio of 113 green to 228 intermediate green to 116 white was obtained in the F_2.

LINT-PERCENTAGE AND LINT-INDEX CHARACTERS

The writer (*16*) has discussed the lint-percentage and lint-index characters and their reaction to environmental influences. The method of determination has been described by Meloy (*15*).

McLendon (*14*), Kottur (*11*), Kulkarni and Khadilker (*12*), Thadani (*22*), Texas Agricultural Experiment Station workers (*20, pp. 47 and 48*), Ware (*23, 25, 26*), and O'Kelly and Hull (*17*) have published results regarding the mode of inheritance of lint-index and lint-percentage characters in cotton. In studies in which both parental lines were upland cottons with fuzzy seed, the results, as far as can be ascertained, are similar to those obtained in the analysis of quantitative characters. In studies in which one parental line was a high-lint-percentage, fuzzy-seed upland and the other parental line was a low-lint-percentage, naked-seed upland, the investigators have found a segregation in which it appeared that lint percentage was controlled by a single factor pair. In these cases the segregation was that of basic seed-fuzz genes. Thadani (*21, 22*), Kearney and Harrison (*10*), Carver (*2*), Griffee and Ligon (*4*), and Ware (*25, 26*) have reported that naked-seed upland cottons differ from the fuzzy-seed uplands by one factor pair to which has been allotted the symbols F^nf^n by Hutchinson and Silow (*9*). Texas workers (*20, p. 48*) and Ware (*25, 26*) have shown that high lint values are associated with the presence of fuzz and sparse lint with naked seed.

RELATION OF GREEN LINT AND LINT INDEX

Hull (7) has called attention to the association between lint color and lint percentage of cotton in the F_2 and backcross populations of a cross between a green-lint strain having a low lint percentage and several white-lint strains having higher lint percentages, and has suggested that the factors that affect lint color are modifiers of lint percentage. Ginning data obtained by the writer and his associates in conducting lint-color inheritance studies provide a verification of this finding.

The purpose of this study was (1) to further demonstrate the relation between the green-lint factor and the lint-index values and (2) to attempt to determine the type of association that exists.

Lindstrom (*13*) has suggested that one of the most direct means of testing whether the inheritance of quantitative characters depends upon the same hereditary mechanism as does the inheritance of qualitative characters is to determine the linkage between these two kinds of characteristics.

There are relatively few published records of the linkage relations between quantitative characteristics and marker genes. Anderson (*1*) has tabulated the summary of work by Sax with weight of beans, of that by Lindstrom with weight of tomatoes and number of rows in corn, and of that by Smith with corolla size in tobacco. Twenty-two marker genes and six quantitative characters were investigated in these studies, and it is noted that linkage was found in all but possibly one of the cases. Hutchinson (*8*) found that lint index in Asiatic cotton is about 6 percent greater in plants with white corollas than in

plants with yellow corollas. Ware (*25*, *26*) reported that low lint-index values in upland cotton were associated with naked seed and that the lint indexes of okra-leaf segregates are lower than those of broad-leaf segregates in the F_2 population of a cross between okra-leaf and broad-leaf lines.

The demonstration of the relation between the green-lint factor and the lint-index values is rather simple. It can be accomplished by growing the F_2 and backcross generations of a cross between a white-lint, high lint-index cotton and a green-lint, low lint-index cotton and determining the significance of the difference between the mean lint-index values of the lint-color phenotypic classes. If the mean value for the white F_2 class is significantly higher than the corresponding value for the green F_2 class, evidence of the association of the green-lint factor and the lint-index values is provided.

The determination of the type of association that exists between a marker gene and a quantitative characteristic is not simple. It consists of determining to what extent the relation is due to linkage and to what extent it is due to pleiotropism.

If the relation is entirely the result of genetic linkage, it must be assumed that the lint index is affected by one or more factors that are located on the same chromosome as the green-lint gene. Such an assumption does not preclude or require the existence of lint-index factors in the other linkage groups. Except for the possibility of a very close linkage between major lint-index genes and the lint-color genes, each of the color phenotypes of the cross should segregate for the lint-index characteristic because of the free assortment of the chromosomes that do not carry basic lint-color genes and because of the chromosome segmental interchanges in the green-lint linkage group. Thus, it should be possible to obtain, in later generations, green-lint plants with lint indexes that, to some extent, approach the index of the white parent.

If the relation between the green lint and the lint index is due entirely to pleiotropism, it must be assumed that the gene that is responsible for the differences in lint color is also responsible for at least a part of the lint-index differences of the two parental lines. Grüneberg (*5*) has distinguished between "genuine pleiotropism," in which the gene produces its manifold effect through different primary effects, and "spurious pleiotropism," in which a single primary effect results finally in manifold effects. Anderson (*1*) has discussed pleiotropism as a hindrance to character recombination in the F_2, and Silow (*19*) has referred to the association of glabrousness and lintlessness in Asiatic cottons as being pleiotropic effects of the same gene. If lint index is affected to any extent by a pleiotropic effect of the basic gene for green lint, the variation of the lint-index values of the green-lint progenies would be restricted by the depressing effect of the green-lint gene; whereas the white-lint progenies of the cross would segregate for the lint-index character independently of the depressing effect of the basic lint-color gene.

MATERIALS AND METHODS

From the foregoing analysis, it follows that a study designed to determine the extent of the association of green lint and lint index, and the type of association, consists of making comparisons in regard

to the lint-index value between the phenotypes of hybrid populations, between the parental lines, and between the hybrid phenotypes and the parental lines.

Two varieties of upland cotton (*Gossypium hirsutum* L.) were used in these studies: Arkansas Green Lint (low lint index) and Half and Half white line (high lint index). The lines had been self-pollinated for several generations prior to the beginning of the study, and they were planted and inbred each season during the study to provide samples for comparison with the F_1, F_2, and F_3 phenotypic classes. In the F_1 generation the plants were backcrossed to each of the parental lines. Seeds from self-fertilized bolls for propagation and seed cotton from open-pollinated flowers for laboratory analyses were obtained from each F_1 plant.

The following year the two backcross and the F_2 populations were grown. Flowers on each of the F_2 plants were self-pollinated to provide seed for propagation of the F_3. Seed cotton from open-fertilized bolls was obtained from each backcross and F_2 plant for laboratory analyses.

During the next season, F_3 progenies from F_2 green, intermediate-green,[5] and white plants were grown. Samples of seed cotton were collected from each plant.

Laboratory analyses consisted of classifying each of the samples for lint color and of determining the lint-index value. There were three principal lint-color classifications: Green, intermediate green, and white. The green-lint phenotypes were divided into two subclasses (G_1 and G_2), and the intermediate-green-lint phenotypes into three subclasses (G_3, G_4, and G_5). The evidence that differences between subclasses within a phenotypic class are due to segregation and recombination of secondary genes is satisfactory.

For the purpose of studying the relation between the lint color and the abundance of lint produced on an individual seed, the seed index and lint percentage are not essential. It was revealed in preliminary analyses that, while the two parental lines differed significantly in regard to the seed index, the segregation in the F_2 plants for lint color was independent of the seed-index characteristic, and that the lint-percentage characteristic in these studies is associated with the lint colors in the same way as is the lint-index character.

RESULTS AND DISCUSSION

The frequency and the range and mean lint-index values of the lint-color phenotypes of the F_1 backcross (BC), F_2, and F_3 populations and of the corresponding parental lines are presented in table 1. Differences and significances of differences between values for lint index of comparable classes are given in table 2. The significances were determined by calculating the t values, according to a method suggested by Fisher (*3*) for cases in which no pairing of samples is possible and the number of samples is not the same for the two series of observations, and by comparing the obtained values with the t values required for the 5-percent and 1-percent levels of significance.

[5] Throughout this paper the term "intermediate green" refers to all segregates heterozygous for the basic factor pair *Lg*, *lg*, and is not confined to those heterozygotes with an intensity of pigmentation that is approximately half way between the intensities of the two parents.

TABLE 1.—*Frequency and range and mean lint-index values of the lint-color phenotypes of the F_1, F_2, BC_1, and F_3 populations and of the corresponding parental lines*

Generation	Phenotype	Frequency	Lint index	
			Range	Mean
			Grams	*Grams*
F_1	Intermediate green	37	4. 43–5. 97	5. 13
Comparable P_1	Green	18	2. 30–3. 00	2. 75
Do	White	19	[1] 4. 83–9. 20	7. 45
BC (F_1 × G)	Intermediate green	24	2. 94–4. 94	4. 05
BC (F_1 × W)	do	16	3. 00–5. 85	4. 88
F_2	Green	113	1. 95–4. 74	3. 01
F_2	Intermediate green	228	2. 30–5. 64	4. 36
F_2	White	116	3. 82–7. 83	5. 77
Comparable P_1	Green	16	2. 13–3. 06	2. 63
Do	White	30	[2] 4. 31–8. 49	6. 97
F_3 (from IG F_2)	Green	38	2. 37–3. 37	2. 98
Do	Intermediate green	83	3. 34–5. 65	4. 51
Do	White	26	3. 89–7. 87	5. 71
F_3 (from G F_2)	Green	80	1. 70–3. 80	2. 93
F_3 (from W F_2)	White	70	4. 17–8. 34	5. 75
Comparable P_1	Green	8	2. 44–3. 06	2. 66
Do	White	30	[2] 4. 31–9. 16	6. 97

[1] Only 1 plant below 5.50 gm.
[2] Only 1 plant below 5.75 gm.

PARENTAL LINES

The original cross was made between green-lint and white-lint stocks with mean lint-index values of 2.75 and 7.45 gm. respectively. The parental green-lint stock was quite uniform, the range between the lowest and the highest value being 2.30 to 3.00 gm., or only 0.70 gm. The parental white-lint stock was considerably more variable, the range being 4.83 to 9.20 gm. Only one plant, however, had a lint-index value below 5.50 gm. The difference between the lint-index values of the two parental lines is highly significant, as shown in table 2.

TABLE 2.—*Differences and significance of differences in lint index of comparable classes of the F_1, F_2, BC_1, and F_3 populations and of the corresponding parental lines of the cross green lint × white lint*

Generation	Phenotypic class having—		Difference [1]
	Higher lint index	Lower lint index	
			Grams
F_1	Intermediate-green F_1	Parental green	2. 38**
	Parental white	do	4. 70**
	do	Intermediate-green F_1	2. 32**
BC	Intermediate-green (F_1 × white)	Intermediate-green (F_1 × green)	.83**
F_2	White F_2	Green F_2	2. 76**
	Intermediate-green F_2	do	1. 35**
	White F_2	Intermediate-green F_2	1. 41**
	Green F_2	Parental green	.38*
	Parental white	White F_2	1. 20**
F_3	Intermediate-green F_3 from intermediate-green F_2	Green F_3 from intermediate-green F_2	1. 53**
	White F_3 from intermediate-green F_2	do	2. 73**
	do	Intermediate-green F_3 from intermediate-green F_2	1. 20**
	Green F_3 from intermediate-green F_2	Parental green	.32
	Parental white	White F_3 from intermediate-green F_2	1. 26**
	White F_3 from white F_2	Green F_3 from green F_2	2. 82**
	Green F_3 from green F_2	Parental green	.27
	Parental white	White F_3 from white F_2	1. 22**

[1] *Statistically significant, $P<0.05$. **Highly significant, $P<0.01$.

F_1 POPULATION

The 37 F_1 plants were classified as intermediate-green lint and had a mean lint-index value of 5.13 gm., which was intermediate between the lint-index values of the parents, indicating lack of dominance. The differences between the lint-index values of the green-lint parent and the F_1 and between the white-lint parent and the F_1 are each highly significant.

BACKCROSS POPULATIONS

Backcross populations consisted of the progenies of the $F_1 \times$ green-lint parent and of the $F_1 \times$ white-lint parent. Information regarding the relation of the lint-color and lint-index characteristics is afforded by the comparison of the lint-index values of the intermediate-green phenotypic classes of the two backcrosses. Theoretically, the two classes should be homologous in regard to the basic gene for the green-lint color. If the lint index is controlled by multiple factors, the $F_1 \times$ green genotypes should have a preponderance of lint-index genes from the green-lint parent, and the $F_1 \times$ white genotypes should have a preponderance of lint-index genes from the white-lint parent. In table 1 it is shown that the mean lint-index value is 4.05 gm., and the range 2.94 to 4.94 gm., for the intermediate-green class of the $F_1 \times$ green backcross, and 4.88 gm., with a range of 3.00 to 5.85 gm., for the intermediate-green class of the $F_1 \times$ white backcross. The mean difference between the two groups is 0.83 gm. and is highly significant.

There was considerable difference in the intensity of the green pigment in the two classes, the $F_1 \times$ green being much the more intense. Two alternative relationships are suggested: (1) The secondary genes that modify the expression of the green-lint factor in doing so modify the lint index, or (2) there is a linkage between the genes that affect lint color and the genes that affect the lint index.

F_2 POPULATION

The F_2 population consisted of 457 plants and segregated into three phenotypic classes, green, intermediate green, and white, approximately in a ratio of 1:2:1. The mean lint-index value of the green-lint class was 3.01 gm.; of the intermediate class, 4.36 gm.; and of the white class, 5.77 gm. There is considerable overlapping of lint-index values of the three phenotypes, the ranges for the green, intermediate green, and white classes being 1.95 to 4.74, 2.30 to 5.64, and 3.82 to 7.83 gm., respectively. The parental strains were continued, and comparisons with the F_2 segregations may be made. The mean lint-index value of the parental green-lint stock was 2.63 gm., with a range of 2.13 to 3.06 gm., and that of the parental white stock was 6.97 gm., with a range of 4.31 to 8.49 gm. It was noted that only one plant of the parental white line had a lint index value of less than 5.75 gm.

A study of the F_2 section of table 2 reveals that the differences in the lint-index values between the white and the green, the intermediate-green and the green, and the white and the intermediate-green classes of the F_2 were 2.76, 1.35, and 1.41 gm., respectively. Each of these differences is highly significant. The significant mean differences between the three phenotypic classes of the F_2 population verify

the association between the lint-index character and the gene affecting the green-lint character.

Some information regarding the association of the two characters is supplied by comparing the green-lint parental line with the green-lint F_2 population, having mean lint-index values of 2.63 and 3.01 gm., respectively, and by comparing the white lint parental line with the white-lint F_2 population, having mean lint-index values of 6.97 and 5.77 gm., respectively. The difference of 0.38 gm. between the green parent and the green F_2 is small and barely significant, whereas the difference of 1.20 gm. between the white parent and the white F_2 is highly significant, as shown in table 2. The Half and Half variety, used as the white-lint parent in this cross, is characterized by a higher lint-index value than most other upland varieties, as it is, no doubt, the product of 50 years of breeding and selection for a high-ginning outturn variety. Even if the suppressing effect of the green-lint gene were eliminated from the green-lint stock, this variety (Arkansas Green Lint) still would be expected to have a lower lint index than Half and Half.

On the basis of the results obtained in these studies, it is suggested that the green-lint parental line possesses low lint-index genes that segregate and recombine apparently independently of the basic green-lint gene. When these genes are combined with the white-lint character, replacing high lint-index allelomorphs, the lint index of the white F_2 is lower than that of the white parental line. Variation in the lint-index values of the white F_2 plants would be due, to some extent, to the segregation of the lint-index factors. At the same time, the mean lint-index value of the green F_2 should have been considerably higher than that of the parent green, since the high lint-index genes contributed by the white-lint parent would combine with the green-lint characteristic, replacing low lint-index allelomorphs of the green-lint parent. However, it is shown in table 2 that the difference between the lint-index value of the green F_2 and that of the parental green plants is very small and barely significant. It may be assumed that segregation took place as outlined, and that the expression of the new gene combination was suppressed to approximately the same level as the expression of the complex of the green parent, directly or indirectly, by the genes affecting the lint color.

F_3 POPULATIONS

The progeny of the intermediate-green F_2 plants segregated into three classes; green, intermediate green, and white, with mean lint-index values of 2.98, 4.51, and 5.71 gm., respectively. There was some overlapping of the intermediate-green and the white segregates. The difference between the mean lint-index values of the intermediate-green and green phenotypes is 1.53 gm.; between the white and green phenotypes, 2.73 gm.; and between the white and intermediate-green phenotypes, 1.20 gm. (table 2). Each of these differences is highly significant. The association between the lint-index character and the factor pair affecting the green-lint character is again demonstrated.

The parental lines were propagated in order that comparisons between the mean lint-index values of these lines and the respective F_3 phenotypes could be made. The difference between the parental green and green F_3 from the intermediate-green F_2 is only 0.32 gm.,

and the obtained t value of 1.57 is less than that required for a significance equivalent to odds of 19 to 1. The results are similar to those obtained in the F_2; and it is again suggested that, if high lint-index genes were acquired by recombination, their expression was prevented by the presence of some effect associated with the basic gene for green lint.

All of the F_3 green-lint segregates in the progeny of the F_2 intermediate-green plants were one grade less intense than were the plants of the parental line. Similar results were noted in the F_2 progeny. It is thus shown that a dilution of the green pigment is not always associated with an increase in the lint-index value. In the discussion of the backcross population it was stated that it is possible that the secondary genes that modify the expression of the green-lint factor in doing so modify the lint-index characteristic. The differences in intensity of the pigmentation of the $F_1 \times$ white and $F_1 \times$ green intermediate-green phenotypes were much greater than the differences between the parental green and the F_2 green or the parental green and the F_3 green. The intermediate-green phenotypes from the $F_1 \times$ white backcross were very dilute. In most plants the green pigmentation was confined to a small part of the sample of seed cotton, the remainder of the sample appearing to be completely devoid of green coloration. The intermediate-green phenotypes from the $F_1 \times$ green backcross produced seed cotton in which the green pigmentation was considerably more intense than the samples obtained from the intermediate-green F_1.

A comparison of the parental white line and the white-lint progeny of the intermediate-green-lint F_2 reveals a highly significant difference of 1.26 gm. and provides additional support to the deduction that the lint-index value of the white F_2 segregates can be decreased by factors obtained from the green-lint stocks in the absence of the basic green-lint gene.

In addition to the progenies of the F_2 intermediate-green-lint plants, progenies of the green-lint and white-lint F_2 plants were grown; in no progeny was there segregation for the basic lint-color factor pair. As shown in table 1, the mean lint-index values were 2.93 and 5.75 gm., respectively. By comparing the green F_3 from the green F_2 with the parental green (table 2), it was found that a difference in lint-index value of 0.27 gm. was not significant. The F_3 white-lint progeny of the F_2 white-lint plants was compared with the white-lint parental line, and a highly significant difference of 1.22 gm. in favor of the parental line was obtained.

EVIDENCE OF "SPURIOUS PLEIOTROPY"

Comparisons of the F_2 or F_3 green-lint phenotypes with the green-lint parent reveal little or no significant difference in mean lint-index value. Comparisons between the F_2 or F_3 white-lint phenotypes and the white-lint parental lines reveal that the F_2 and F_3 whites have a significantly lower lint-index value than does the parental white line. These associations suggest that in the segregation and recombination of lint-index genes low lint-index factors from the green-lint stock are combined with the white-lint gene, resulting in the F_2 white segregates having a lower mean lint index than the white parental line. In this segregation and recombination, it might logically be expected

that high lint-index factors from the white-lint stock would be combined with the green-lint gene. However, the data indicate that the green-lint gene suppresses the expression of the new complex to approximately the same lint-index level as the expression of the complex of the green-lint parental line.

While the multiple-factor theory of inheritance of quantitative characteristics has not been proved in these studies, no hypotheses were evolved that would, if verified, disprove the theory. If the lint-index value in white cottons is affected by a large number of factor pairs, it is quite probable that some of these genes are located in the green-lint linkage groups. However, this linkage relationship fails to account entirely for the association of the green-lint and lint-index characteristics. In the F_2 population it is relatively easy, merely by selecting green-lint phenotypes, to recover segregates that approach the parental lint-index combination of the green-lint stock. However, the recovery of combinations that approximate the white lint is much more difficult.

The evidence favors the theory that the green-lint and lint-index association is largely due to the pleiotropic effect of the basic green-lint gene. It appears that the effect is one of "spurious pleiotropy," the green-lint gene affecting the pigmentation in the fiber wall. In turn the pigmentation physiologically affects the lint index.

EFFECT OF GREEN PIGMENTATION

If the theory of "spurious pleiotropy" accounts for the association of the green-lint character and low index values, it must be assumed that the presence of the pigmentation affects the development of the fiber initials into normal fibers.

The diameter, wall thickness, estimated area, and maturity of fiber from the parental lines, the F_1 population, and the F_2 segregates were determined. The results are given in table 3.[6]

TABLE 3.—*Major diameter, minor diameter, ratio of circularity, wall thickness, and maturity of fibers from the parental lines, F_1 population, and F_2 phenotypes of the cross green lint × white lint*

Generation	Phenotype	Major diameter	Minor diameter	Ratio of circularity [1]	Wall thickness	Estimated area	Mature fibers
		μ	μ		μ	μ²	*Percent*
P_1	Green	26.30	2.00	13.15	0.72	52.9	0
P_1	White	21.45	7.50	2.86	3.30	157.0	80
F_1	Intermediate green	22.17	5.79	3.83	2.42	129.0	40
F_2	Green	24.70	2.55	9.67	.85	62.2	0
F_2	Intermediate green	27.50	3.55	7.75	1.25	97.0	0
F_2	White	20.50	7.65	2.68	3.00	152.9	60

[1] Ratio of circularity = major diameter/minor diameter.

There was a marked difference in the cross-sectional characteristics of the green-lint and white-lint parental lines. The most outstanding difference was the wall thickness, the values being 0.72μ and 3.30μ for the green-lint and white-lint lines, respectively. The ratios of circularity for the two lines were 13.15 and 2.86, respectively.

[6] The values given in table 3 were determined by T. L. W. Bailey, Jr., associate cotton technologist, Agricultural Marketing Administration, U. S. Department of Agriculture, who also made the photomicrographs used in preparing figures 1 and 2.

The values for the F_1 intermediate green were intermediate between those for the parents, the wall thickness being 2.42μ and the circularity ratio being 3.83.

Although the F_2 intermediate green did not have as thick walls as the F_1 intermediate green, the association between the cross-section characteristics and the green pigmentation is obvious. The green-lint F_2 and white-lint F_2 samples were comparable to the respective parental types in regard to wall thickness and the ratio of circularity.

The values representing maturity were obtained by inspection of photomicrographs of the cross sections and comparison with a series of photomicrographs depicting different percentages of mature fibers, based upon extensive observations and actual counting of immature fibers according to the standard procedures proposed by Richardson, Bailey, and Conrad (*18*). The parent white and the F_2 white compared satisfactorily with average commercial cottons with respect to maturity. The F_2 was somewhat lower in maturity than the parent white, and the low lint percentage of certain F_2 white segregates may have been due to the higher percentage of immature fibers. Apparently the green possesses factors for immaturity other than those associated with green pigmentation. However, these factors are of less consequence than the green factor. The F_1 and F_2 intermediate-green plants produced fibers that were decidedly immature.

In figures 1 and 2, the differences in regard to diameter, ratio of circularity, and wall thickness are illustrated. Cross sections of fiber from the green-lint parent and the white-lint parent (fig. 1, *A* and *B*) show the small minor diameter and the thin wall of the fiber from the green-lint parent as compared with the larger minor diameter and thicker wall of the fiber from the white-lint parent. Cross sections of the F_1 intermediate-green fiber (fig. 1, *C*) show that the minor diameter and wall thickness are intermediate between those of the two parents.

Cross sections of fibers from the green-lint, intermediate-green-lint, and white-lint F_2 phenotypes (fig. 2, *A*, *B*, and *C*) show that the fibers from the recovered green and white phenotypes compare closely with the respective parental lines in regard to minor diameter and wall thickness and that the fibers from the F_2 intermediate-green phenotype are intermediate between the green and the white phenotype.

The data shown in table 3 and in figures 1 and 2 indicate that the presence of the green pigmentation in the cell wall results in immature fibers with thin walls and relatively small minor diameters. The individual fibers of the green segregates weigh less than those of the white segregates, and consequently the lint index for the green fiber is less.

SUMMARY AND CONCLUSIONS

Crosses were made between two strains (Arkansas Green Lint and Half and Half white lint) of upland cotton (*Gossypium hirsutum* L.), characterized respectively by green lint, low lint index, and white lint, high lint index.

The F_1 was intermediate green with a mean lint-index value that was intermediate between those of the two parents.

In the backcross generations, satisfactory 1:1 ratios of green to intermediate green or intermediate green to white were obtained. In

FIGURE 1.—Cross sections of cotton fibers from (*A*) green-lint parent, (*B*) white-lint parent, (*C*) F_1 intermediate-green lint of green × white cross. × about 1,000.

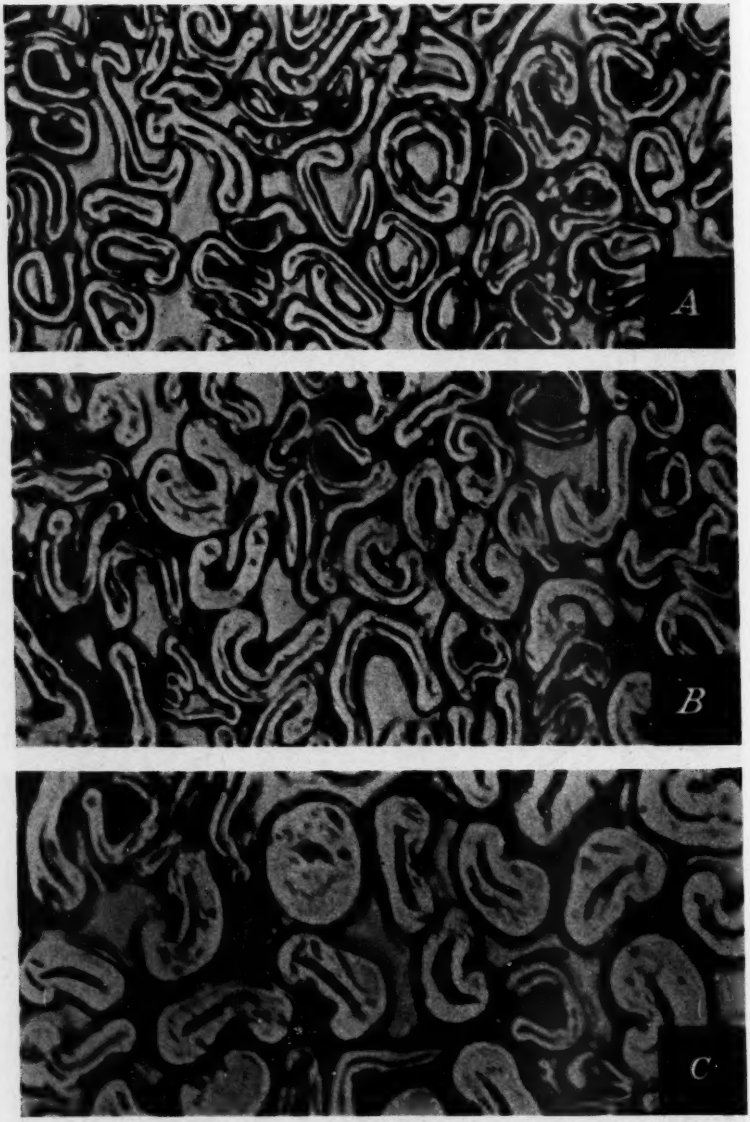

FIGURE 2.—Cross sections of cotton fibers from F_2 phenotypes of the cross green lint × white lint: *A*, Green-lint segregate; *B*, intermediate-green-lint segregate; *C*, white-lint segregate. × about 1,000.

the F_2 generation, satisfactory 1 : 2 : 1 ratios of green, intermediate green, and white were obtained. The previous conclusion that the characteristic is controlled by one genetic factor pair is confirmed.

In the analysis of samples from the backcross, F_2, and F_3 phenotypes it was shown that green lint and low lint index are very closely associated. Linkage fails to account for the association. When high lint-index factors from the white-lint stock are combined with the green-lint gene, the green-lint gene suppresses the expression of the lint-index genes. The green-lint and lint-index association is largely due to the pleiotropic effect of the basic green-lint gene.

The effect appears to be one of "spurious pleiotropy," the green-lint gene affecting the pigmentation in the fiber wall. In turn, the pigmentation prevents the development of the fibers. The result is that green fibers have thin walls and small minor diameters and consequently very low lint-index values.

It is indicated that green-lint types with a lint index approximating that of commercial white cottons cannot be developed through breeding.

Perhaps the "spurious pleiotropic" effects of factors may account for many cases of so-called antagonism between characteristics of domesticated plant species.

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HERITABLE RELATION OF WAX CONTENT AND GREEN PIGMENTATION OF LINT IN UPLAND COTTON[1]

By CARL M. CONRAD, formerly *senior cotton technologist, Agricultural Marketing Administration,* and J. WINSTON NEELY, *geneticist, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture*[2]

INTRODUCTION

The wax content of cotton lint has been the subject of a number of investigations. Lecomber and Probert (*8*)[3] studied cottons originating in different parts of the world in order to determine the extent to which their origins could be identified by the content and properties of wax. It was found that cottons could not be identified in this way. Fargher and Higginbotham (*6*) studied scoured cottons with reference to the percentage losses sustained and the amount of certain noncellulose constituents, including waxes, retained after various kiering treatments. Ahmad and Sen (*1*) were interested in the possible influence of wax content on the classer's judgment of "silkiness" or "harshness." Fargher and Probert (*4*), Clifford and Probert (*2*), and Fargher and Higginbotham (*5*) have reported that the different components of the wax from the lint in American and Egyptian cottons are highly complex mixtures of various classes of waxy substances.

As early as 1911, Knecht (*7*) reported that Egyptian raw cotton from which the wax had been removed was difficult to handle in the drawing and spinning processes. Excessive amounts of waste, irregular results, and a tendency for the fiber to adhere to the rollers were encountered. In spinning finer count yarns, excessive breakage, which was also observed in the weaving processes, occurred. Knecht suggested that benefits in the manufacturing process and in the strength of the material might result from supplementing the low natural wax content of ordinary cotton fiber with additional oily or waxy substances. This has been done occasionally by spraying mineral oils and other substances on the fiber at different stages in the progress of manufacture.

Conrad (*3*) has shown that whereas the wax content of most cotton lint varies within the range of from 0.4 to 0.7 percent, that of Arkansas Green Lint cotton, based upon the dry weight, reached the high value of 17 percent. This finding suggested the possibility of studying the influence of a high natural wax content on the spinning quality of cotton.

The Arkansas Green Lint cotton is not suitable for commercial production because of its small yield and low ginning outturn. Neely (*9*) has shown that the green pigmentation and low ginning outturn are "spurious pleiotropic" effects of the same gene and that it is very unlikely that green-lint strains with lint percentages approximating

[1] Received for publication June 16, 1942. Cooperative investigations between the Agricultural Marketing Administration and the Bureau of Plant Industry, U. S. Department of Agriculture, and the Mississippi Agricultural Experiment Station. The senior author is now with the Southern Regional Research Laboratory, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, New Orleans, La. When the investigation was made this work was a part of the Agricultural Marketing Service. On February 23, 1942, that Service was merged with the Surplus Marketing Administration and the Commodity Exchange Administration to form the Agricultural Marketing Administration.

[2] The authors wish to acknowledge their indebtedness to Dr. J. O. Ware, senior agronomist, Bureau of Plant Industry, who made helpful suggestions in regard to the manuscript, and to Meyer D. Silverman, scientific aide, Agricultural Marketing Administration, who made the wax determinations.

[3] Italic numbers in parentheses refer to Literature Cited, p. 312.

the commercial white cottons can be developed through breeding. In many cases the green-lint cotton would not be suitable for commercial production because of its color and fiber shortness. Since no other cotton is known that has the high wax content of the green-lint strains, interest has been manifested in the possibility of combining, through hybridization and subsequent selection, the high wax content of the green-lint strains with the lint color and other desirable characteristics of the commercial white-lint strains. Thus, information regarding the type of heritable relation that exists between the wax content and the green-lint character is of considerable importance. It was the purpose of this investigation to study these relations.

MATERIAL AND METHODS

The samples employed in these studies were collected at Stoneville, Miss., during the 1938 and 1939 crop season and were used in studies to determine the relation of green lint to lint index in upland cotton. Two strains of cotton were used in these studies: Arkansas Green Lint (high wax content) and Half and Half white lint (low wax content). These strains were self-pollinated for several generations prior to the beginning of the study.

The methods employed for selecting parent plants and for self- and cross-pollination have been described by Ware (*10*). The procedure followed in obtaining samples from parental lines and of F_1, backcross, F_2, and F_3 segregations and in classifying the samples, as well as the mode of inheritance of the green-lint characteristics, have been outlined by Neely (*9*).

Lint samples of the 1938 growth from all plants of a given lint-color classification and parental line or progeny were composited and thoroughly mixed for wax-determination samples.[4] Because of the large number of plants in the 1939 growth, only about half of the plants of a given lint-color classification and progeny were composited for each determination.

After compositing and thoroughly mixing, duplicate $10 \pm .01$-gm. samples of lint were weighed out and placed, without grinding, in the extraction compartments of 50 by 250 mm. Soxhlet extractors, provided with 300-ml. distillation flasks. Thimbles were not used. The material was compacted so that all fiber would be covered with the condensed liquid before the latter siphoned over. Then, 250 ml. of 95-percent alcohol was poured into the cotton; a portion siphoned over and collected in the flask below. The condenser was attached, the water turned on, and the extraction continued for 6 hours from the time of boiling. At the end of this period the flame under each flask was turned out at the moment (within 1 to 5 minutes) that the alcoholic extract remaining in the flask amounted to about 100 ml.

The amount of wax contained in the alcoholic solution was determined by a method to be described elsewhere by Conrad.[5] Briefly, it consists of the transfer of the hot alcoholic solution to 100 ml. of chloroform in a 1,000-ml. separatory funnel, the addition of sufficient distilled water to cause separation, and the removal of the chloroform layer from the bottom. The aqueous layer is then washed three times

[4] Although, from the genetical standpoint, it would be desirable to determine, separately, the wax content of the lint from each plant of the progenies, the amount of work involved was prohibitive for the amount of time that could be allotted.

[5] CONRAD, C. M. A NEW EXTRACTION METHOD FOR THE DETERMINATION OF TOTAL WAX IN COTTON FIBER. (In preparation.)

with 50-ml. portions of chloroform, after which the combined chloroform extracts are washed once with distilled water. The sugars and other water and alcohol-soluble substances pass more or less completely to the water layer, while the waxy substances are retained in the chloroform layer. The chloroform-soluble fraction is then evaporated and dried in a tared weighing bottle at 105° C., weighings being made at 30-minute intervals until the loss is not more than 1 mg.

Separate determinations of moisture were made, and the wax content was computed to the oven-dried weight.

RESULTS

The percentages of wax of the lint-color phenotypes of the F_1, backcross, F_2, and F_3 populations and of the corresponding parental lines from the 1938 and 1939 growths are given in tables 1 and 2, respectively. The percentage of wax in each case represents the mean of two or more closely agreeing values.

TABLE 1.—*Wax content of lint-color phenotypes of F_1 and first-generation backcross progenies of a cross between Half and Half white lint and Arkansas Green Lint cotton and of the corresponding parental lines; 1938 growth, families 1, 2, and 3*

Generation	Family	Progenies of—	Wax content of lint-color phenotypes		
			White	Intermediate green	Green
			Percent	*Percent*	*Percent*
P_1	1	P_1 parents	0.63		15.04
BC	1	F_1 × green-lint parent		3.42	13.92
BC	1	F_1 × white-lint parent	.60	2.11	
F_1	1	F_1 (white × green)	.54	3.96	12.32
P_1	2 and 3	Parents of F_1	[1].56		13.12
F_1	2 and 3	Green- × white-lint parents		[2] 2.17	

[1] Lint from only 1 plant represented.
[2] Lint from 2 families, 2 and 3, combined.

TABLE 2.—*Wax content of lint-color phenotypes of first-generation backcross, second-generation backcross, F_2, and F_3 progenies of a cross between Half and Half white lint and Arkansas Green Lint cotton and of the corresponding parental lines; 1939 growth, families 1, 2, and 3*

Generation	Family	Progenies of—	Wax content of lint-color phenotypes		
			White	Intermediate green	Green
			Percent	*Percent*	*Percent*
P_1	1	Family 1 parents	0.48		12.64
BC_2	1	Intermediate BC on green	.53	2.84	12.53
BC_2	1	Intermediate BC on white	.58	2.36	12.13
F_2	1	Green F_2 phenotype			12.90
F_2	1	Intermediate F_2 phenotype	.56	2.26	11.36
F_2	1	White F_2 phenotype	.66		
P_2	2	Family 2 parents	.63		12.82
BC	2	F_1×white-lint parent	.59	1.95	
F_2	2	F_1 (white×green)	.51	2.40	12.42
P_3	3	Family 3 parents	.61		12.82
BC	3	F_1×white-lint parent	.55	1.92	
F_2	3	F_1 (white×green)	.54	2.52	12.12

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PARENTAL LINES

The original cross was made between white-lint and green-lint stocks with wax percentages of 0.63 and 15.04, respectively. Inbred progenies of the parental lines were grown each season, and very little variation in the wax content was found. The range was from 0.48 to 0.63 percent for the white-lint parental line and from 12.64 to 15.04 percent for the green-lint line. The difference in the wax content of the two stocks is striking.

THE F_1 POPULATION

The F_1 plants were classified as intermediate green, and the wax content was found to be 2.17 percent, as shown in table 1. Thus, there is not complete dominance in regard to the wax content, but the F_1 value is nearer that of the low-wax-content parent.

THE BACKCROSS POPULATION

Backcross populations consisted of the progenies of the $F_1 \times$green-lint parent and of the $F_1 \times$white-lint parent crosses. Information regarding the relationship of the lint-color and wax-content characteristics is afforded by the comparison of the wax percentages of the intermediate-green phenotypic classes of the two backcrosses. Theoretically, the two classes should be homologous for the green-lint color basic-factor pair. The $F_1 \times$green genotypes should have a preponderance of wax-content genes from the green-lint parent and the $F_1 \times$ white genotypes should have a preponderance of wax-content genes from the white-lint parent, if the wax content is controlled by multiple factors. It is shown in table 1 that the mean wax content is 3.42 percent for the intermediate-green class of the $F_1 \times$green backcross and 2.11 percent for the intermediate-green class of the $F_1 \times$white backcross, or a difference of 1.31 percent.

The $F_1 \times$green intermediate-green progenies were considerably more intensely green than were the $F_1 \times$white intermediate-green progenies. It is indicated that the secondary genes that modify the expression of the green-lint factor also modify the wax content.

The wax content of the intermediate-green segregates of the backcross $F_1 \times$white of families 2 and 3 is 1.95 and 1.92 percent, respectively. The white-lint segregates of the two families have average wax percentages of 0.59 and 0.54 percent.

The progeny of the intermediate-green phenotypes of the backcrosses segregated into 1 white: 2 intermediate green: 1 green. The wax content of these classes was 0.53, 2.84, and 12.53, percent, respectively, for the backcross to the green parent and 0.58, 2.36, and 12.13 percent, respectively, for the backcross to the white parent. The wax content of the comparable white-lint and green-lint parental lines is 0.48 and 12.64 percent, respectively. The difference between the wax content of the white and green segregates and the corresponding parental type is apparently of no consequence. The wax-percentage differences between the three phenotypes of the second backcross generations verify the association between the wax-content character and the gene affecting the green-lint character.

THE F_2 POPULATIONS

The F_2 populations, consisting of one family in 1938 and two families in 1939, were grown. Segregation for the lint-color character in each family corresponded satisfactorily to the expected 1:2:1 ratio

of white, intermediate green, and green phenotypes. The association of high wax content and green lint is shown. The wax percentages for the white, intermediate-green, and green phenotypes, as shown in tables 1 and 2 for family 1, were 0.54, 3.96, and 12.32, respectively; for family 2, 0.51, 2.40, and 12.42; and for family 3, 0.54, 2.52, and 12.12. Comparable white-lint parental lines for the three families had wax percentages of 0.63, 0.63, and 0.61, respectively; and comparable green-lint parental lines for the three families had wax percentages of 15.04, 12.82, and 12.82, respectively. It is possible that the wax content of the F_2 green-lint segregates was significantly lower than that of the green-lint parental lines. Again it is indicated that the secondary genes that modify the expression of the green-lint factor pair also modify the wax content, since the F_2 green-lint segregates were less intensely green than the green-lint parental lines.

THE F_3 POPULATIONS

The progeny of the intermediate green F_2 plants segregated into three classes: green, intermediate green, and white, with mean wax percentages of 0.56, 2.26, and 11.36, respectively. The association between the wax content and green-lint pigmentation is again demonstrated.

The parental lines were propagated in order that comparisons between the wax percentages of these lines and the respective F_3 phenotypes could be made. The difference between the parental white and the F_3 white from the F_2 heterozygote is 0.08 percent and is probably of no consequence. Similarly, the difference between the parental green and the F_3 green from the F_2 intermediate green is rather small in comparison with the difference between the two phenotypes.

The F_3 white progeny from the F_2 white phenotypes had a wax content of 0.66 percent as compared with a wax content of 0.48 percent for the parental white. There is some indication that small differences in wax content which are not associated with differences in lint pigmentation may occur. The difference between the wax content of the F_3 green progeny from th F_2 green phenotypes and the green-lint parental line is small and probably of no significance.

SUMMARY AND CONCLUSIONS

The total wax content of lint from Half and Half white lint and Arkansas Green Lint upland cottons and of the F_1, backcross, F_2, and F_3 populations of crosses between the two strains was determined. The samples represent two consecutive crop years and three sets of families representing three different crosses. The wax content of the white-lint parent varied within the low limits of 0.48 to 0.63 percent and that of the green-lint parent between the much higher limits of 12.64 to 15.04 percent.

The F_1 was intermediate green with a mean wax content between that of the two parents but closer to that of the white parent.

In the analyses of samples from the backcross, F_2, and F_3 phenotypes, it was shown that the green lint and high wax content were closely associated.

While there is no evidence against the relationship being one of genetical linkage, it is indicated that the relationship is probably a physiological one and that the genetic factor that affects the green

pigmentation also affects the wax content. Previous work has shown that the presence of green pigmentation in the fiber cell wall is associated with a suppressed development of fiber initials into mature fibers. Just what is the interrelationship of cause and effect between high wax content, green pigmentation, and suppressed development of fiber wall remains to be clarified.

The results are not conclusive, but it is indicated that there is little likelihood of combining the high wax content of the green-lint strain with the white lint of commercial cotton. It is possible that the genetic behavior in crosses between other green-lint strains and the same or other white-lint varieties would be different. These studies are being extended to include such crosses.

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ASCORBIC ACID CONTENT OF STRAINS OF SNAP BEANS [1]

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INTRODUCTION

In 1936 a program of breeding hardy, productive, high-quality snap beans for the southern United States was undertaken at the United States Regional Vegetable Breeding Laboratory, Charleston, S. C. Although the desirability of incorporating high ascorbic acid content, as well as other desirable qualities, in the lines of snap beans being developed was evident, satisfactory methods for the determination of this vitamin on large numbers of samples were not available when the work was initiated. Since then, however, rapid and accurate methods have been devised for the determination of ascorbic acid; these have made possible investigations in the plant-breeding program that were not feasible a few years ago. The subjects in the present study were relatively finished varieties of snap beans, some of which are pure lines, but the techniques used herein are also applicable to studies of the ascorbic acid content of segregating lines.

MATERIALS AND METHODS

In 1940, 46 strains of beans (*Phaseolus vulgaris* L.) derived from crosses of U. S. No. 5 Refugee (or closely related lines) with Stringless Black Valentine, Bountiful, and Brittle Wax were released to the 13 collaborating Southeastern States for trial. Some of these strains when released were quite uniform; others were segregating for seed color, pod types, and other factors. While these beans were being grown by some of the collaborating States, they were also being grown at Charleston, S. C. For the crops of 1940, rapid chemical methods for the determination of ascorbic acid content were not available. However, preliminary work by Morell (*9*) [2] resulted in an improvement for use here of Bessey's (*1*) modification of Mindlin and Butler's (*8*) method. The crops of 1941 were analyzed for ascorbic acid content at each of four harvests of each crop.

The strains were arranged in a 7 by 7 lattice square with 4 series (replicates), according to a method of Weiss and Cox (*13*). For convenience the strains were numbered 1 to 49, with 9, 48, and 49 representing the commercial varieties Brittle Wax, Bountiful, and Stringless Black Valentine, respectively, and the other numbers, which are preceded by VBL,[3] the 46 crosses previously mentioned. All strains had previously been observed to mature for market within a day or two of each other under Charleston conditions. Plots were single rows 3 feet apart and 32 feet long. Locally grown seed of the

[1] Received for publication July 30, 1942. This work was performed under an allotment from the Special Research Fund authorized by Title I of the Bankhead-Jones Act of June 29, 1935.

[2] Italic numbers in parentheses refer to Literature Cited, p. 324.

[3] VBL indicates a strain originated by the U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C.

46 strains originated by the United States Regional Vegetable Breeding Laboratory was used throughout the experiment, but western-grown seed of the 3 commercial varieties was used. For the most part good stands were obtained.

Spring and fall crops of pods were each harvested four times. A complete harvest of pods never required more than 4 hours. Only pods at market or canning stages were included, thus eliminating variability due to immaturity or overmaturity of pods. Samples were placed in bags, stored at 36° F. within 30 minutes after picking, and analyzed within 48 hours. Twenty-five grams of unselected whole pods was used for analysis; broken and damaged pods were excluded.

Two pickings of leaves were made of each crop—the first a few days after the second picking of pods and the second a few days after the fourth picking of pods. Leaf harvests were begun at about 8:30 a. m. and were completed in 2 hours. Samples of 25 gm. were picked, and 10 gm. was used for analysis.

The following procedure, developed by Morell (*9*), was used in analyzing the samples. After the stems and tips had been removed, the pods were broken into four parts. The petioles were removed from the leaves. A suitable sample of pods or leaves, weighed to the nearest 0.1 gm., was added to 100 ml. of 3-percent metaphosphoric acid in a blender container and mixed at high speed for 2 minutes. This produced a fine suspension, which was filtered through a No. 12 Whatman paper. A convenient aliquot of the filtrate was transferred to a 50-ml. volumetric flask and adjusted to pH 3.6 by the addition of 0.25 ml. of a sodium citrate buffer per milliliter of aliquot. The mixture was then made to volume with a citrate-phosphate buffer. The pH of the final solution was maintained at 3.6 $\pm$0.1. A 5-ml. portion of this solution was transferred to an absorption tube containing 5 ml. of the indicator (sodium 2,6-dichlorobenzenoneindophenol), and the photometric reading was made with an Evelyn photoelectric colorimeter with filter No. 520. Blank readings were obtained with 5 ml. of the citrate-phosphate buffer. The analyses were made in groups of 24, and approximately 100 determinations were completed in a day. The percentage of water in the plant tissue, which must be taken into account in calculating the ascorbic acid content, was determined by drying weighed samples in an electric oven at 103° C. for 18 hours.

The manganese in the soil was determined by the ammonium persulfate method (*12, p. 305*); an Evelyn photoelectric colorimeter with a No. 540 filter was used. Soil samples were taken with a soil auger to a depth of 14 inches from three places in each plot. The samples were taken immediately after the fourth harvest of pods in the spring.

During the period from planting to the first spring harvest in 1941 there occurred one of the severest droughts on record for this area. The beans were irrigated three times, but the heat was so intense that at times many of the plants drooped because they were not able to take up moisture as fast as needed. Many local commercial, nonirrigated bean plantings were abandoned, but the beans at the Vegetable Breeding Laboratory produced a normal crop, with only very slight nonrecovery from the drooping observed.

During the fall of 1941 the temperatures were much above normal for this area until about the time of the third harvest of pods. The fourth harvest of pods and the second picking of leaves occurred after the temperature had dropped to 28° F. for a short while. Only pods and leaves showing no frost damage were used in the chemical analyses. This planting was irrigated twice, but other beans grown without irrigation gave fair yields.

RESULTS

PRELIMINARY AND INCIDENTAL INVESTIGATIONS [4]

A study was made to determine whether there was any relation between stage of maturity and ascorbic acid content. For this work six varieties representing both small- and large-podded types were used. Within each strain small size represented a relatively immature pod, large size a slightly overmature one, and medium the size ordinarily harvested for canning or marketing. Equal numbers of records were not available for each strain. There were no significant differences between large, medium, and small pods within a strain. The ascorbic acid content for 58 pods of each size averaged 17.2, 16.9, and 17.6 mg. per 100 gm., respectively.

The effect on ascorbic acid content of storage at 36° F. and at 98 to 100 percent relative humidity was studied partly in combination with the stage-of-maturity experiment and partly on other materials. There was a slight but nonsignificant increase in ascorbic acid content up to 72 hours, but at 96 hours there was a highly significant decrease of 6.94 mg., or 38 percent, from that of the 72-hour period (table 1).

TABLE 1.—*Effects of storage at 36° and 70° F. on ascorbic acid content of snap beans*

Average storage temperature (° F.)	Ascorbic acid content per 100 gm. (fresh-weight basis) after storing for the number of hours specified [1]				
	None [2]	24–48	72	96	168
	Milligram	*Milligram*	*Milligram*	*Milligram*	*Milligram*
36 [3]	17.87	17.46	18.38	11.44**	
70 [4]	16.25	15.04	10.98**		9.31**

[1] ** indicates that content was highly significantly different from that of samples that were analyzed immediately after picking (column 2).
[2] The difference in entries in this column is due to the difference in the varieties tested in the 2 groups.
[3] 15 determinations at each period.
[4] 30 determinations at each period.

In another experiment the effects of storage at room temperature (70° F.) on ascorbic acid content were studied for various lengths of time up to 1 week. There was a slight decrease at 48 hours after harvest, and a sharp and significant one at 72 hours. At 168 hours there was a further decrease to somewhat more than half the initial amount, but the content was not significantly lower than at 72 hours (table 1). The storage room was rather favorable for holding the beans in good condition, since it was cool and well ventilated and the beans were not exposed to sunlight. In this experiment relative humidity was not recorded.

[4] Parts of the preliminary investigations were carried out by S. A. Morell, formerly of the Division Fruit and Vegetable Crops and Diseases.

A study was undertaken in the fall of 1941 to determine the distribution of ascorbic acid in various parts of the plant. Plants were selected from rows from which no pods or leaves had been harvested, and determinations were made on roots, pods, leaves, and stems. The large, sparsely leaved variety Stringless Black Valentine and the small, abundantly leaved strain VBL 19 were used. The roots were found to contain about half as much ascorbic acid as the pods, the leaves four to five times as much as the pods, and the stems as much as or nearly twice as much as the pods, depending upon the variety (table 2).

TABLE 2.—*Ascorbic acid content of various parts of bean plants*

[Average of 3 determinations per variety for each part]

Variety or strain	Ascorbic acid content per 100 grams (fresh-weight basis) of—			
	Roots	Pods	Leaves	Stems
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
Stringless Black Valentine	10.62	26.38	101.19	27.93
VBL 19	9.11	19.22	104.71	37.74
Average	9.87	22.80	102.95	32.84

A further study was made to determine the range of ascorbic acid content of a widely divergent group of beans. The lowest content per 100 gm. (8.13 mg.) was found in the pods of a promising hybrid, while the highest content (36.55) was found in those of the field bean Idaho No. 1 Mosaic Resistant Great Northern. In general, tender, high-quality strains or varieties, such as Blue Lake (12.23), U. S. No. 5 Refugee (19.40), and Kentucky Wonder (15.99), were low in ascorbic acid content, while the more fibrous strains or varieties had a higher content. An exception was the fibrous Sure Crop Wax, which was low in ascorbic acid content (13.83).

MAIN INVESTIGATIONS

The average ascorbic acid content of the 49 strains or varieties grown in the spring ranged from 19.1 to 28.7 mg. per 100 gm. per strain (table 3), while the values for the same strains grown in the fall were from 16.2 to 26.4. The averages for fall and spring combined ranged from 17.7 to 27.5 mg. The highest ranking variety (No. 48) was Bountiful; the lowest was the hybrid strain VBL 6 (table 3).

There was a slight but highly significant decline in the ascorbic acid content of the spring crop of pods from the first two pickings to the third and fourth (table 4), but in the fall the first picking had an average of less than half that of the first spring picking (12.16 vs. 24.41 mg. per 100 gm.), and the difference is highly significant. The ascorbic acid content of the pods from the second fall picking was significantly below the average for the corresponding spring picking. The third fall picking was not significantly different from the third spring picking, but the fourth was highly significantly above any of the other seven pickings. The coefficients of variability were 13.34 percent for the spring and 16.80 percent for the fall pickings. The average ascorbic acid content was 23.39 mg. per 100 gm. for spring pickings, and none of the four series (replicates) diverged more than 4.96 percent

TABLE 3.—*Ascorbic acid content of pods and leaves of 49 strains or varieties of snap beans, Charleston, S. C., 1941*

Cross or variety	Pods						Leaves					
	Spring		Fall		Both		Spring		Fall		Both	
	Ascorbic acid content per 100 gm. (fresh-weight[1] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight[2] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight[3] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight[2] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight[2] basis)	Rank	Ascorbic acid content per 100 gm. (fresh-weight[1] basis)	Rank
	Milligrams		*Milligrams*		*Milligrams*		*Milligrams*		*Milligrams*		*Milligrams*	
VBL 1	20.0	46	17.4	46	18.7	46	121.6	26	144.8	43	133.2	40
VBL 2	21.9	35	18.5	40	20.2	41	109.4	46	160.3	22	134.9	36
VBL 3	21.3	38	18.3	41	19.8	42	100.6	49	153.0	35	126.8	46
VBL 4	21.4	37	17.7	44	19.5	43	109.8	44	155.6	30	132.7	41
VBL 5	20.3	43	21.6	14	20.9	34	107.5	48	159.9	23	133.7	39
VBL 6	19.1	49	16.2	49	17.7	49	108.4	47	142.5	46	125.4	47
VBL 7	19.4	48	17.6	45	18.5	47	120.8	28	150.2	37	135.5	34
VBL 8	23.1	29	20.6	20	21.9	24	125.6	17	147.9	40	136.8	32
Brittle Wax (No. 9)	25.6	9	21.3	16	23.5	11	132.8	4	155.2	34	144.0	17
VBL 10	23.5	25	22.9	7	23.2	13	118.2	35	164.7	15	141.4	20
VBL 11	22.2	33	22.8	8	22.5	20	121.3	27	157.8	26	139.6	25
VBL 12	25.2	10	21.3	15	23.3	12	124.5	21	155.5	31	140.0	24
VBL 13	24.6	16	23.7	5	24.1	8	112.9	43	161.4	19	137.2	31
VBL 14	23.2	28	19.1	33	21.1	30	129.0	8	182.6	2	155.8	2
VBL 15	20.1	44	21.9	12	21.0	33	123.8	22	164.6	16	144.2	16
VBL 16	20.0	45	16.4	48	18.2	48	117.4	37	147.4	41	132.4	43
VBL 17	27.9	3	20.3	21	24.1	7	122.9	24	169.3	8	146.1	13
VBL 18	21.2	41	19.9	26	20.5	37	120.2	29	155.3	33	137.7	29
VBL 19	26.6	5	19.2	32	22.9	15	109.8	45	170.5	7	140.1	23
VBL 20	23.3	27	20.2	23	21.8	25	118.7	33	149.3	39	134.0	38
VBL 21	23.6	23	19.8	29	21.7	26	126.4	15	461.0	20	143.7	18
VBL 22	22.1	34	18.8	38	20.4	38	116.0	38	147.2	42	131.6	45
VBL 23	22.6	31	18.2	42	20.4	40	123.6	23	162.9	18	143.2	19
VBL 24	25.1	11	20.3	22	22.7	19	119.0	32	156.7	27	137.8	28
VBL 25	19.6	47	18.1	43	18.8	45	119.6	30	144.3	45	132.0	44
VBL 26	23.6	24	18.6	39	21.1	31	117.4	36	150.9	36	134.2	37
VBL 27	24.1	20	19.8	28	21.9	23	113.3	40	156.6	29	134.9	35
VBL 28	24.8	15	24.4	4	24.6	4	127.2	11	155.5	32	141.3	21
VBL 29	25.0	13	22.6	9	23.8	9	113.1	41	137.2	48	125.2	48
VBL 30	20.4	42	21.0	19	20.7	35	113.1	42	134.1	49	123.6	49
VBL 31	25.0	12	23.2	6	24.1	6	122.4	25	184.3	1	153.4	3
VBL 32	21.5	36	19.9	27	20.7	36	114.9	39	159.6	24	137.3	30
VBL 33	26.3	6	19.7	30	23.0	14	129.4	7	165.0	14	147.2	11
VBL 34	24.2	19	18.9	37	21.6	27	125.4	18	163.2	17	144.3	15
VBL 35	24.0	21	20.1	24	22.0	22	131.2	5	150.0	38	140.6	22
VBL 36	23.9	22	19.0	35	21.4	28	134.2	3	161.0	21	147.6	9
VBL 37	21.2	39	19.7	31	20.4	39	127.6	10	166.2	12	146.9	12
VBL 38	24.6	17	21.0	18	22.8	17	129.5	6	173.2	4	151.4	5
VBL 39	25.7	8	20.0	25	22.9	16	118.5	34	157.9	25	138.2	26
VBL 40	22.5	32	21.7	13	22.1	21	125.7	16	169.1	9	147.4	10
VBL 41	27.1	4	22.0	11	24.6	5	127.6	9	168.8	10	148.2	8
VBL 42	24.4	18	21.2	17	22.8	18	134.7	2	168.5	11	151.6	4
VBL 43	23.1	30	19.1	34	21.1	32	126.5	14	172.1	5	149.3	6
VBL 44	23.4	26	19.0	36	21.2	29	135.8	1	179.8	3	157.8	1
VBL 45	24.9	14	22.4	10	23.7	10	125.0	19	166.0	13	145.5	14
VBL 46	28.2	2	26.4	1	27.3	2	124.9	20	139.9	47	132.4	42
VBL 47	21.2	40	17.4	47	19.3	44	127.2	12	144.6	44	135.9	33
Bountiful (No. 48)	28.7	1	26.3	2	27.5	1	126.8	13	170.7	6	148.8	7
Stringless Black Valentine (No. 49)	25.8	7	25.4	3	25.6	3	119.2	31	156.6	28	137.9	27
Average	23.39		20.42		21.91		121.44		158.58		140.0	
Significant difference:												
5-percent point[4]	2.53		2.78		1.88		14.69		24.28		13.45	
1-percent point	3.35		3.64		2.49		19.45		32.15		18.67	

[1] Average of 4 harvests (16 determinations).
[2] Average of 8 harvests (32 determinations).
[3] Average of 2 harvests (8 determinations).
[4] Significant difference for strain means.

from this amount; in the fall the average was 20.42 mg., and none of the series diverged more than 5.53 percent. If the first picking in the fall is excluded, the average for fall is 23.17, almost identical with the spring value.

In both spring and fall there was a decline in ascorbic acid content of the leaves from the first picking (a few days later than the second picking of pods) to the second picking (a few days later than the fourth picking of pods). The average level of ascorbic acid in leaves was much lower in the spring than in the fall (121.4 mg. vs. 158.6 mg. per 100 gm.) (table 4). The coefficients of variability for spring and fall were 10.55 percent and 13.36 percent, respectively. In the spring the most divergent replicate differed by 6.09 percent from the mean, while in the fall the most divergent replicate differed by 7.19 percent. The average content of the leaves for the average of spring and fall harvests varied from 123.6 mg. to 157.8 mg. per 100 gm.

TABLE 4.—*Average ascorbic acid content of 49 strains or varieties of snap beans in relation to weather during the 48 hours before picking, Charleston, S. C., 1941*

[196 samples at each picking date]

PODS

Crop and picking date	Ascorbic acid content per 100 gm. (fresh-weight basis)	Weather conditions		
		Sunshine	Average temperature	Rainfall
Spring crop:	*Milligrams*	*Hours*	*° F.*	*Inches*
May 27	24.41	26	75	0
June 2	24.61	12	80	0
June 8	21.96	25	77	0
June 14	22.60	17	78	0
Average (784 samples)	23.39			
Fall crop:				
Oct. 21	12.16	2	75	0.32
Oct. 28	21.47	16	70	0
Nov. 3	21.32	13	70	.27
Nov. 10	26.73	20	53	0
Average (784 samples)	20.42			
Significant difference M_{196}:				
5-percent level	.68			
1-percent level	.89			
Significant difference M_{784}:				
5-percent level	.39			
1-percent level	.45			

LEAVES

Spring crop:				
June 5	128.64	21	64	0
June 17	114.22	15	49	0
Average (392 samples)	121.44			
Fall crop:				
Oct. 31	179.79	11	64	0
Nov. 12	137.38	15	49	0
Average (392 samples)	158.58			
Significant difference M_{196}:				
5-percent level	3.84			
1-percent level	5.05			
Significant difference M_{392}:				
5-percent level	2.72			
1-percent level	3.57			

The average amount of ascorbic acid in the spring crop of pods was 19.26 percent of that in the leaves. In the fall this value dropped to 12.88 percent. On the basis of absolute amounts the fall average of pods was 87.26 percent of the spring amount, but the spring average for leaves was only 76.58 percent of the fall average.

In both spring and fall there was a nonsignificant negative correlation of the ascorbic acid content of the first and second harvest of pods with that of the first harvest of leaves, −.034 and −.007. In the case of the third and fourth harvest of pods and the second harvest of leaves there were positive correlations of +.444** [5] and +.302*.

The manganese content of the soil was positively correlated with the ascorbic acid content of the spring crop of pods, +.173*, and negatively correlated with the content of the spring harvest of leaves, −.136. The manganese content of the soil varied from 55 to 329 p. p. m., with a mean content of 167 p. p. m. for the plots on which the spring crop was grown.

DISCUSSION

Preliminary investigations indicated that stage of maturity was not associated with large differences in the ascorbic acid content of pods. This finding confirmed that of Mack, Tapley, and King (*7*), who reported that there was not much difference between immature and overmature pods, and that the least ascorbic acid occurred in beans of the mature marketable stage. The differences between the three classes were much less than those found by Mack, Tapley, and King. Most of the analyses of pods reported in this investigation (table 3) were made on pods of marketable maturity.

Storage at 36° F. was much more effective in conserving ascorbic acid than storage at room temperature (70°). However, up to 48 hours there was not much difference in conservation of values between refrigeration and room temperature. After 48 hours there was a sharp drop in the ascorbic acid content of pods held at room temperatures, but under refrigeration the sharp drop did not come until after 72 hours. In a week at room temperature pods lost approximately half their original ascorbic acid content. Mack, Tapley, and King (*7*), using four varieties of snap beans, three sets of temperatures, and 6 days of storage, found markedly greater losses for both storage periods and temperatures than were found in these experiments. Humidity may have some bearing on this, since Mack, Tapley, and King stored at 60 to 70 percent relative humidity, and in the experiment reported here the relative humidity for the 36° storage groups was 98 to 100 percent. That humidity has a bearing on the retention of ascorbic acid is indicated by Harris, Wissmann, and Greenlie (*5*). They indicated that the rate of destruction of the vitamin in six vegetables, including snap beans, stored at an average relative humidity slightly less than 65 percent averaged about 64 percent greater than in corresponding samples stored at an average relative humidity of 93 percent.

Analyses of various parts of the bean plant indicated that roots do not store much ascorbic acid and that stems and pods have much less than leaves. These results agree quite well with those obtained

[5] Values marked ** are statistically significant by odds of 99 : 1 or more; values marked * are significant by odds of 19 : 1 or more, but less than 99 : 1.

by Reid (*10*) in a study of the distribution of ascorbic acid in cowpea plants.

The small correlations obtained between manganese content of the soil and ascorbic acid content of pods and of leaves indicated that in this experiment the manganese content was not a limiting factor although varying from 55 to 329 p. p. m. of soil. Hester (*6*) found a positive correlation between the manganese content of soil and the ascorbic acid content of tomatoes.

Varieties with large pods ranked highest in ascorbic acid content, but some of the smaller podded varieties also ranked high. Large-podded varieties used in this study also have relatively large seeds, and the pods tend to fill out more quickly than those of the small-podded strains. The varieties ranking highest in the Mack, Tapley, and King study (*7*) were those showing the same tendencies. The first four ranking strains all have large, market types of pods, while VBL 41, with the rank of 5 for a combination of both fall and spring, has a smaller, canning type of pod. The flat-podded types, Bountiful (No. 48) and VBL 46, ranked 1 and 2 and the oval-podded types, Stringless Black Valentine (No. 49) and VBL 28, ranked 3 and 4; while round types were not represented until 5, 6, and 7, with VBL 41, VBL 31, and VBL 17, respectively. That flatness is not closely associated with high ascorbic acid content was indicated by the fact that the flat strain VBL 14 had a rank of 30.

Leafiness is a characteristic that might be expected to influence the ascorbic acid content of pods, since in citrus fruits it has been found that those fruits which are more exposed to sunlight on the outer parts of a tree have a greater ascorbic acid content than the more shaded fruits from the inner parts of the tree (*3*, *4*). VBL 46 and Bountiful (No. 48) have a few large leaves and the pods are much exposed to sunlight, whereas VBL 19 and VBL 41 have an abundance of leaves which exclude a good deal more sunlight than the first two mentioned. The average (spring and fall) ascorbic acid content of the pods of VBL 46 and Bountiful was much greater than that of VBL 19 and VBL 41, but in the spring there was not much difference. In the early fall when there was less sunshine but hotter weather the ascorbic acid content of VBL 19 fell off sharply, but later in the fall when temperatures were lower and light intensity was further reduced, it approached that of Bountiful.

A study of the rankings of the pods of the 49 strains or varieties studied (table 3) indicates that on the average a strain which had a relatively high level of ascorbic acid in the spring likewise had a high level in the fall, and vice versa. Bountiful (No. 48), for instance, ranked 1 in the spring and 2 in the fall, while VBL 6 ranked 49 in both seasons. However, there were important exceptions to this general rule. VBL 19, which ranked 5 in the spring, ranked 32 in the fall, and VBL 5, which ranked 43 in the spring, ranked 14 in the fall. Possibly some of these interactions of ascorbic acid content with season may be part of the response of certain strains to length of day. Possibly, also, if ascorbic acid is needed in the metabolism of the bean plant, the leaves may utilize more and those of different varieties may utilize different quantities of it during periods of adverse weather. That ascorbic acid is needed in the metabolism of the plant is indicated by Reid (*11*). When the pods of VBL 19 ranked 5 in the spring, the leaves (table 3)

ranked 45; but, when the pods ranked 32 in the fall, the leaves ranked 7 in ascorbic acid content.

VBL 1 to 8, Brittle Wax (No. 9), and VBL 15 to 27, inclusive, are wax-podded strains; all others are green-podded. These 22 wax-podded strains had on an average much less ascorbic acid in the pods than the 27 green-podded strains, but that high content is not closely associated with the green-podded condition was indicated by the high rank attained by VBL 17 (a wax-podded type), which ranked 7 for spring and fall combined.

The two strains which had the highest average ascorbic acid content in the pods were strikingly different in the ascorbic acid content of the leaves. Bountiful (No. 48), which ranked 1 for content of pods, ranked 7 for leaves, while VBL 46, which was 2 for content of pods, ranked 42 for leaves (based on the averages for spring and fall combined). Considering all strains in the spring, there was a very small nonsignificant negative correlation (−.034) between first and second harvest of pods and first harvest of leaves. In the fall the correlation for these items was −.007. Between the third and fourth harvests of pods with second harvest of leaves the correlation was +.444** for spring and +.302* for fall.

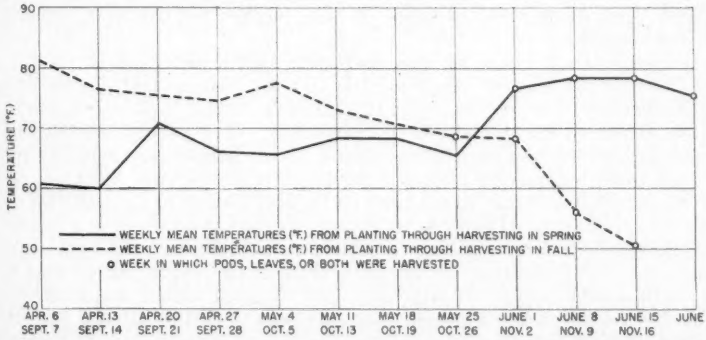

FIGURE 1.—Mean temperatures during the growing of two bean crops at Charleston, S. C.

Bountiful has light-green leaves and pods, Stringless Black Valentine has dark leaves and dark pods, and VBL 46 has medium-dark leaves and pods. For the total of all harvests the pods of these varieties ranked 1, 3, 2 and the leaves 7, 27, 42, respectively, in ascorbic acid content. This indicates that it is doubtful whether any significant relation exists between the intensity of green pigmentation of the strains and the amount of ascorbic acid in the pods.

The distribution of the temperatures for the spring and fall crops was strikingly different (fig. 1). The average temperatures based on weekly means were almost identical for the entire growing period, i. e., approximately 70° F. in both cases. Despite the great difference in weather conditions preceding the first pod harvest in the spring and that in the fall, both the lowest and the highest average ascorbic acid content were found in the fall crop, and (excluding the first fall pod harvest average) the spring and fall means were nearly identical (23.39

and 23.17, respectively). It appears that the ascorbic acid content of the pods was not much influenced by the weather during the period from planting to harvest. Mack, Tapley, and King (7) reported no significant seasonal effect over a period of 3 years. Considering the differences in soil and climatic conditions between Geneva, N. Y., and coastal South Carolina, some of the results are strikingly similar. For instance, Mack, Tapley, and King (7) found a value for U. S. No. 5 Refugee identical with that found here (19 mg. per 100 gm.); for Blue Lake they found a value of 11 mg., while that for Charleston was 12 mg.

A report of the sunshine, temperature, and rainfall during the 48-hour period just prior to each harvest (table 4) was obtained from the local station of the United States Weather Bureau, 8 miles away. It is noticeable that the October 21 harvest with its very low average was preceded by a 48-hour period during which there were only 2 hours of sunshine. Since carbohydrate substances are known to be low during periods of low light intensity, it might reasonably be expected that a low concentration of ascorbic acid would be found in the pods during a period of low light intensity extending over a period of 2 days. That temperature during this period was not an especially important factor is indicated by the fact that, although the temperature was lowest for the highest ranking harvest of pods, it was highest for the next highest ranking harvest.

Although the average for the pods was significantly lower for fall than for spring, the reverse was true for the leaves, and the lowest value obtained for the leaves in the fall was higher than the highest values obtained for the leaves in the spring. The second picking of leaves in both spring and fall averaged much below the first picking in each case, suggesting that the problem is one of translocation in the course of which the leaves tend to become somewhat exhausted of their ascorbic acid. The data in table 2 suggest that there is a translocation gradient from leaves to stems to pods to roots. The problem is somewhat complicated by the fact that in the spring there was a very dry windy period during May and June in which water losses from the leaves were so high that temporary wilting of the leaves occurred despite adequate applications of irrigation water. Although the fall crop was grown at a slightly higher average temperature, no wilting of leaves was observed at any time. There is also the additional complication that in the spring the days were becoming longer, while in the fall they were becoming shorter.

The coefficients of variability for ascorbic acid content of pods for spring and for fall were less than the corresponding coefficients of variability for yield of snap beans from the same plants and less than those usually found for snap bean and seed bean yields at the Charleston station. The soil on which the crops were grown was moderately uniform, and there was not much variation in ascorbic acid from one replicate to another. Most of the differences found could be accounted for by differences in variety and season. In no case did any picking or any combination of pickings of pods or of leaves fail to show significant varietal differences. The first picking of pods in the fall differed significantly from all other pickings and was the major factor in the significant seasonal difference observed. These results

contrast strikingly with those obtained by Currence (*2*) in experiments with tomatoes grown under field conditions in Minnesota. He found some interaction of variety and season, but in general the varieties that were high in ascorbic acid in the spring were also high in the fall, and vice versa. It is probable that the coefficients of variability would have been somewhat less if all the 46 VBL strains had been homozygous. Many of these strains were still segregating for various horticultural characteristics, and consequently any genetic variability for ascorbic acid content that may have occurred would be confounded with the random error component of the variance analysis.

SUMMARY AND CONCLUSIONS

In preliminary experiments it was found that stage of maturity—immature, marketable, and overmature—made only very slight differences in the ascorbic acid content of snap beans. Marketable pods were used in all the varietal experiments.

All pods and leaves used in the varietal experiments were analyzed either immediately or within 48 hours after storage at 36° F. Storage at 36° prevented loss of ascorbic acid much better than storage at room temperature, but there was not much difference in the two storage treatments until after 48 hours. The leaves had the highest ascorbic acid content, followed in order by the stems, pods, and roots, suggesting a translocation gradient.

In an experiment carried out in the fall of 1941 with several strains and varieties of beans, it was found that in general the higher quality varieties, such as Blue Lake, U. S. No. 5 Refugee, and Kentucky Wonder, had much less ascorbic acid than more fibrous beans, such as the field bean Idaho No. 1 Mosaic Resistant Great Northern.

In the major experiment reported, 46 hybrid strains and 3 commercial varieties were arranged in a lattice square in 4 replicates, and ascorbic acid determinations were made from each of 4 pickings of pods from a spring planting and the same number from a similar fall planting. Determinations were made on leaves corresponding as closely as possible in each case to second and fourth pickings of pods.

There were significant varietal differences for each picking and for the average of all pickings. The strains tested varied from 19.1 mg. per 100 gm. to 28.7 in the spring, from 16.2 to 26.4 in the fall, and from 17.7 to 27.5 for fall and spring combined. The variety with the highest ascorbic acid content was Bountiful (No. 48), although there was no significant difference between it and hybrid VBL 46, which resembles it. All other strains contained significantly less ascorbic acid than Bountiful on the basis of the complete set of data for pods. Although high-quality strains contained significantly less ascorbic acid than Bountiful, some were sufficiently close to justify the belief that it is possible to combine high quality with a very high ascorbic acid content.

In general, if a strain ranked high or low in ascorbic acid in the spring, it had a similar rank in the fall, although there were enough exceptions to indicate that some strains would rank much higher under one set of conditions than under another.

There were very significant differences between strains in respect to the ascorbic acid content of their leaves. The leaves were always

much higher in ascorbic acid than the pods. The lowest average content of leaves in the fall was higher than the highest content in the spring.

Consideration was given to ascorbic acid content in relation to size of pod, leafiness of plant, waxiness or greenness of pods, intensity of greenness in leaves and pods, and weather conditions at harvesting time and to correlations between ascorbic acid content of pods and that of leaves.

A small, significant, positive correlation was found between manganese in the soil and the ascorbic acid content of the combined harvests of pods for spring, and a small negative, nonsignificant correlation between manganese in the soil and the ascorbic acid content of the combined harvests of leaves for spring.

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